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PRINCIPAL INVESTIGATOR: William L. Hanson, Ph.D.

CONTRACTING ORGANIZATION: The University of Georgia
Research Foundation, Incorporated
Athens, Georgia 30602

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The University of Georgia Research Foundation, Inc. Athens, Georgia 30602 E-MAIL: WLHanson@BellSouth.Net			
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<p>A total of 34 selected compounds, including natural products, some compounds known to be efficacious against other diseases, cholesterol-lowering drugs currently in use in human beings, and pro drugs of the pentamidine type were studied in hamsters at various dosage levels, <u>via</u> various routes and treatment schedules for antileishmanial efficacy against <u>Leishmania (Leishmania) donovani</u>. One of the same compounds (Baycol) was studied for efficacy against <u>Leishmania (Viannia) panamensis</u> and <u>Leishmania major</u> and three other compounds were studied for efficacy against <u>Leishmania (V.) panamensis</u>. Only two of these compounds had any efficacy against visceral leishmaniasis (BN97515 and an analog of Amphotericin B). The activity of the Amphotericin B analog was not as great as Amphotericin B and BN97515 was active but toxic. None were active against cutaneous leishmaniasis.</p> <p>Several topical ointment preparations (containing the same drug but different water concentrations) were highly efficacious in mice against cutaneous lesions caused by <u>Leishmania major</u>.</p> <p>When biopsy cultures obtained by WRAIR from human and canine patients with suspected infections with <u>Leishmania</u> were injected into hamsters and/or mice <u>via</u> various routes, the presence of cutaneous leishmaniasis was confirmed in 38 human patients and the presence of visceral leishmaniasis was confirmed in 8 other patients (5 human beings and 3 canines).</p>			
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FOREWORD

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Introduction

Protozoan parasites of the genus Leishmania are widespread throughout the world with at least 80 countries known to be endemic for these parasites where they cause a complex of visceral or cutaneous diseases in human beings as well as some domestic animals such as dogs (1-3). Since the leishmanias commonly exist as zoonoses, these diseases pose a significant potential threat to the health and performance of military personnel as well as military dogs throughout the extensive endemic areas throughout the world (1, 4-7). Relatively recent publicity in both scientific and other media regarding possible infection with these diseases of personnel involved in Operation Desert Storm has reemphasized the military significance of the leishmanias. Available current information regarding the importance of leishmaniasis as an opportunistic infection in HIV-infected persons living in both endemic and non-endemic areas (8, 9) adds to the importance of these parasites in human health.

The leishmanias are extremely difficult to treat in general and treatment of patients also infected with HIV is especially difficult (10, 11). Although chemotherapy offers the best potential mechanism for the management of these diseases in infected human beings or animals, drugs currently available for use against these parasites are often not satisfactorily effective and are potentially toxic to man and animals. Consequently, better orally administered drugs for the treatment of the leishmanias are much needed.

As a result of the importance of Leishmania to human and animal health, this laboratory has been involved for several years in studies to identify new antileishmanial compounds which are more efficacious against these parasites and less toxic for human beings as well as animals. In these studies we have refined animal model systems for testing potential compounds with antileishmanial efficacy using both visceral (Leishmania (L.) donovani) and cutaneous (Leishmania (V.) panamensis) leishmaniasis in hamsters as well as non-human primates (12-16).

While some success has been achieved in the search for better antileishmanial drugs in these studies (17-21), as well as by studies of others (22), most of these compounds have met with limited success in the field in both immunocompetent and immunocompromised patients. Thus it is necessary to continue the search for better antileishmanial drugs.

Because of the need for better antileishmanial drugs for use in humans and dogs, the primary purpose of this project was to receive potential antileishmanial drugs from officials at the Walter Reed Army Institute of Research (WRAIR) and test these compounds for efficacy against Leishmania in rodents as well as non-human primates when warranted. During part of this project

period this laboratory has also received biopsy cultures obtained by officials at WRAIR from patients suspected of being infected with Leishmania (or possibly some other blood parasite) with two primary objectives: (a) verification of leishmanial infections by injection of these cultures primarily into hamsters or by subinoculation of the cultures into other media; (b) determination of the infectivity of these Leishmania for hamsters and/or mice and obtain preliminary data regarding infections observed thus enabling future in vivo drug sensitivity studies of these isolates in laboratory animals. Data obtained from these studies will be important for the future management of leishmanial infections in patients at WRAIR as well as other patients elsewhere.

Materials and Methods

I. Testing of Potential Antileishmanial Compounds

A. Study of Selected Compounds for Activity Against Experimental Visceral Leishmaniasis

The standard primary visceral test system which has been used in this laboratory for many years was used for the study of some of the compounds involved in this report. In this test system a Khartoum strain of Leishmania (L.) donovani (WR378) was used and the golden hamster (Mesocricetus auratus, Harlan Sprague Dawley, Inc., Indianapolis, Indiana), 50-70 gm, served as the host animal. Suspensions of amastigotes for experimental infection of hamsters were prepared by grinding heavily infected hamster spleens in sterile saline in a Ten Broeck tissue grinder and diluting the suspensions so that 0.2 ml contained approximately 10×10^6 amastigotes. After being tranquilized with 0.3 ml of a Ketamine Hydrochloride/Rompun (Xylazine) solution (16.7 mg/ml and 5.0 mg/ml respectively) each experimental hamster was infected via the intracardiac injection of 0.2 ml of the amastigote suspension.

The testing procedure used was that described by Stauber and his associates (23, 24) as modified by Hanson, et al. (12). On Day 3 following infection, hamsters were divided randomly into experimental groups consisting of a minimum of 6 animals per group, initial group weights were obtained, and administration of test compounds was initiated. Each compound was tested at 2 or 3 drug dosage levels dependent upon the priority rating and nature of the compound.

The vehicle for the test compounds was 0.5% hydroxyethyl cellulose-0.1% Tween 80 (HEC-Tween). Each test group contained six hamsters and received one of the desired drug dosage levels. A control group of six hamsters received the 0.5% HEC-Tween vehicle only and the reference compound, Glucantime®, was given at 3 drug dosage levels (208, 52, and 26 total mg/kg) based on antimony content. Test compounds were administered routinely once daily via various routes dependant on the nature of the compound on Days 3 through 6. Final group weights were obtained on all experimental hamsters on Day 7 and all animals were killed with CO₂, livers removed, weighed, and liver impressions made for enumeration of amastigotes. Subsequently, the total number of parasites per liver was determined as described by Stauber, et al. (23, 24).

In addition to recording body weight changes as a general indicator of toxicity of the test compounds, experimental hamsters were observed for such clinical signs of toxicity as nervous disorders, roughened hair coat, and sluggish activity. Deaths of the animals was also used as an indication of significant drug toxicity.

After determining the ratio of numbers of amastigotes per host cell nucleus, the weight of the organ, and initial and final weights of the hamsters, the raw data was evaluated with a Gateway 2000 microcomputer using a program which calculates percent weight change, total numbers of parasites, mean numbers of parasites per organ, and percent parasite suppression. The computer program then performs linear and non-linear regression analysis and calculates a SD_{50} for active compounds from each of the analyses (drug dosage resulting in 50% suppression of amastigotes). The SD_{50} from the non-linear analysis is used for a comparison of the relative efficacies of the test compounds as well as the relative efficacy of test compounds to that of the reference compound, Glucantime. The linear regression analysis is included only for comparison with the non-linear analysis.

Some modifications were made in the primary visceral test system for the study of some of the compounds covered by this report, namely a series of compounds currently approved for oral use in human beings for treatment of high cholesterol levels, and candidate pro drugs of the pentamidine type. The modifications used involved primarily treatment schedules and in one experiment, mortality was used to evaluate the antileishmanial efficacy of the test compounds. In these studies treatment with cholesterol-lowering drugs was initiated 2 to 7 days prior to infection and continued for one to two weeks following infection. Hamsters were killed and drug efficacy evaluated one day following completion of treatment in these experiments. In one experiment treatment was initiated 3 days prior to infection and continued until death of the hamsters.

In one experiment selected combinations of the cholesterol-lowering drugs were studied. In this experiment BP14986 (Lipitor) was administered to all infected hamsters at a total dosage level of 182 total mg/kg and BP05021 (Lamisil) or BP08044 (Questran) were administered at various dosage levels in combination with Lipitor. Treatment was administered to all groups of hamsters beginning 7 days prior to infection and continuing for 7 days following infection.

In the experiment involving the pro-drugs, pentamidine type, oral treatment of experimental hamsters was initiated 3 days following infection, continued for 7 days, and the efficacy was evaluated one day following completion of treatment.

With the exception of these modifications, the other procedures used in these studies were the same as those for the primary visceral test system.

B. Testing of Compounds for Activity Against Experimental Cutaneous Leishmaniasis

The Primary Cutaneous Test System was used in some of these studies in which Leishmania (V.) panamensis (WR539) was the

experimental parasite and male golden hamsters, 50-70 gm, served as experimental hosts.

Promastigotes for establishing experimental infections in hamsters were grown in Schneider's Drosophila Medium (Hendricks, et al., 25) and quantitated using procedures described previously (Hanson and Roberson, 26). In preparation for infection and weekly during the experiment, the hair was clipped on the dorsal tail head and a commercial depilatory agent applied to the areas to remove the remaining hair. After being tranquilized with 0.3 ml of a Ketamin Hydrochloride/Rompun (Xylazine) solution (16.7 mg/ml and 5.0 mg/ml respectively), each hamster was inoculated via the intradermal route with approximately 1.5×10^7 promastigotes of L. (V.) panamensis near the base of the tail using a 0.25 ml glass syringe equipped with a 30 gauge $\times \frac{1}{2}$ " needle. Each experimental group consisted of six hamsters. Initial body weights were obtained and administration of therapy, via various routes dependant on the nature of the compound, was initiated on Day 19 postinfection, and continued through Day 22 postinfection. Glucantime was included at two dosage levels (832 and 208 total mg/Sb/kg) as the reference compound, and a group of six hamsters received vehicle only (HEC-Tween). Test compounds were administered generally at 320 and 52 total mg/kg.

Lesion area of each experimental hamster was determined one week after completion of treatment with the aid of a template made at WRAIR and calibrated according to the formula $r_1 r_2^{\text{II}}$ where r_1 is the major radius of the lesion and r_2 is the minor radius (Wilson, et al., 27). The mean lesion area of each experimental group was obtained and the percent suppression of lesion size calculated by comparing the mean lesion area of each treated group with that of the group receiving vehicle only using a computer program and a Gateway 2000 microcomputer. The computer program performs linear and non-linear regression analysis and calculates an SD_{50} for each active compound using both analyses. The SD_{50} obtained from the non-linear analyses is used for a rough comparison of the relative efficacies of the test compounds as well as the relative efficacy of each test compound with that of the reference compound, Glucantime. The linear regression analysis is performed for comparison with the non-linear analysis.

In one special experiment Leishmania (V.) panamensis (WR539) and L. major (WR779) were used and Female Balb/c mice, approximately 20 gm (Charles River) served as experimental hosts.

Promastigotes for establishing experimental infections in mice were grown in Schneider's Drosophila Medium (Hendricks, et al., 25) and quantitated using procedures described previously (Hanson and Roberson, 26). In preparation for infection and weekly during the experiment, the hair was clipped on the dorsal tail head and a commercial depilatory agent applied to the areas to remove the remaining hair. After being tranquilized with 0.1 ml of a Ketamine Hydrochloride/Rompun (Xylazine) solution (16.7 mg/ml and 5.0 mg/ml

respectively), each mouse was inoculated via the intradermal route with approximately 1.5×10^7 promastigotes of L. (V.) panamensis or 5×10^6 L. major near the base of the tail using a 0.25 ml glass syringe equipped with a 30 gauge X $\frac{1}{2}$ " needle. Each experimental group consisted of ten mice. Initial body weights were obtained and administration of therapy, via the oral route was initiated on the day of infection, and continued through Day 14 postinfection. A group of ten mice received vehicle only (corn oil). The test compound, Baycol, was administered at 6 mg/kg/day.

Lesion area of each experimental mouse was determined on the day of completion of treatment and one week after completion of treatment as described in the previous paragraph for L. (V.) panamensis in hamsters.

Two other experiments were conducted to determine the antileishmanial efficacy of several topical cream preparations of WR279396 using L. major, Balb/c mice, and special protocols.

In these experiments promastigotes of L. major were grown, harvested, quantitated, hair removed from base of tail, and mice infected as described in the preceding paragraphs. In the first experiment the infection was allowed to progress until the mean lesion area was approximately 250 mm^2 . Mice were then divided into two groups, one of which contained 4 mice and received the placebo (BN85864, mean lesion size 262.5 mm^2) and a second group containing 6 mice which was treated with the test sample (BN85873, mean lesion area 258.3 mm^2).

Treatment began 61 days following infection and continued for 10 days. Test preparations were applied to lesions twice daily with 0.1 ml per 100 mm^2 of lesion using a 1.0 ml syringe. Lesions were cleaned once a day using sterile gauze sponges and liquid soap and water.

Lesion measurements were taken at completion of treatment and days 7, 14, 21, 28, 35, 50, 61, and 70 following completion of treatment. The evaluation of BN85873 treatment (percent suppression) was determined by the formula: mean lesion size of the placebo control group minus the mean lesion size of the group receiving BN85873 divided by the mean lesion size of the placebo control group times 100.

In the second experiment a total of 50 mice were infected and lesions were allowed to grow on all experimental mice for three weeks post infection. Mice were then divided into five groups containing ten mice each having approximately equal mean lesion sizes (62.6 mm^2).

Prior to treatment, initial group body weights were taken as well as final group body weights following the completion of treatment.

Treatment began on Day 21 post infection and continued for 10 consecutive days. One group received the placebo (BP15287, 32.9% water concentration) while the other four groups received either BP15241 (34.0% water concentration), BP15250 (37.0% water concentration, BP15269 (40.4% water concentration) or BP15278 (43.8% water concentration). The test compounds were applied as a salve directly onto the surface of the lesions. Test preparations were thoroughly stirred and applied to lesions twice daily with 0.1 ml per 100 mm² of lesion using a 1.0 ml syringe. While all containers were stirred equally, BP15278 (43.8% water concentration) required the most stirring in order to produce consistency of the salve. Once applied on the lesion, this preparation tended to "run" off of the infected area. All of the other water concentrations including the placebo remained in place on the lesion. Lesions were cleaned once a day using sterile gauze sponges and liquid soap and water.

Lesion measurements were taken one, two, and three weeks following completion of treatment and treatment efficacy evaluated as described in the first of these experiments. All mice were terminated following the three week post treatment measurement.

II. Infectivity of patient biopsy cultures for laboratory animals and verification of the presence of Leishmania.

A variety of biopsy cultures from human patients as well as some canine patients suspected of leishmanial infection were periodically sent to this laboratory from WRAIR for study of infectivity for laboratory animals. When received, each culture was examined microscopically for the presence of flagellates and a portion of each culture was subinoculated into Schneider's Drosophila medium (Hendricks et al., 25). Each of the subinoculated cultures was checked microscopically weekly for a four week period. Positive cultures were transferred to fresh media while negative cultures were terminated after the four week period. The remainder of the culture received from WRAIR was inoculated into hamsters and/or mice as instructed by WRAIR officials. Essentially the same procedures for injection of laboratory animals and the monitoring for the presence of infection were used in this aspect of the work as was described in the preceding section for drug studies. Following is a brief summary of these procedures.

A. Hamsters

Male golden hamsters (Mesocricetus auratus) 40-80 gm were used. Patient cultures were injected into tranquilized hamsters via the intracardial, intraperitoneal, or intradermal route as per WRAIR instructions. Groups of 2-6 hamsters per culture were used.

Hamsters receiving injections via the intracardial route were each given 0.1 - 0.2 ml of the desired culture. Each group was monitored daily for clinical signs and/or death for a period of 6-12

months. If death occurred during this time, impressions were made of the liver, spleen, and bone marrow and cultures seeded with ground spleen homogenate. At the end of the six month period, all surviving hamsters were killed with CO₂ and spleens (occasionally also liver and bone marrow) were removed and ground in Schneider's Drosophila media using a Ten Broeck tissue grinder. The spleen homogenates from each hamster from the group were then combined and a sample inoculated into a single culture flask containing Schneider's Drosophila media, 20% inactivated Fetal Bovine Serum (Gibco BRL, Life Technologies, Grand Island, N.Y.) and 1.0% gentamycin solution (Sigma Chemical Co., St. Louis, MO). These pooled spleen cultures were then examined for the presence of Leishmania and subsequently shipped to WRAIR for their perusal. On occasion the organs of individual animals were cultured separately.

Prior to intradermal inoculations, the hair of hamsters was clipped on the dorsal tail head. Injections of 0.05-0.1 ml of the desired culture was injected into tranquilized hamsters at the base of the tail. Weekly thereafter, hair was removed from the tail head of each hamster using a commercial depilatory agent (Nair, Carter Products, Carter-Wallace, Inc., New York, NY). Hamsters were observed daily for lesion development or other clinical signs and/or death for a period of 6-12 months. When lesions appeared, photographs were taken of all lesions, a hamster with a representative lesion was killed with CO₂, the cutaneous lesion removed, touch preparations of the lesion were made on microscope slides, and the lesion ground in Schneider's Drosophila media, 20% inactivated Fetal Bovine Serum and 1% gentamycin solution. Photographs of all lesions and microscope slides with lesion impressions were forwarded to WRAIR as they were obtained.

Cultures obtained were passaged weekly for 3-4 weeks and a culture was shipped to WRAIR. A duplicate culture from each hamster lesion was passaged weekly thereafter in this laboratory for an additional 17-30 weeks dependant on the growth of the culture and freezing down time of the WRAIR laboratory. When advised that WRAIR had successful frozen isolates, the cultures were discarded. If groups of hamsters remained negative for lesion growth for a period of six months, the group was terminated by CO₂ asphyxiation. In some instances the skin from the original injection area was removed, impression made, and cultures made and forwarded to WRAIR.

B. Mice

One to four Balb/c mice (Harlan Sprague Dawley, Inc., Indianapolis, Indiana) 15-20 gm were injected via the intraperitoneal, intracardial, or intradermal route with 0.1 - 0.2 ml of selected cultures. Following injection of the samples, mice were handled as described for hamsters in the preceding paragraphs receiving intracardial or intradermal injection.

Results and Discussion

A total of 28 new compounds which were selected by officials at WRAIR and forwarded to this laboratory were studied for antileishmanial efficacy against visceral leishmaniasis caused by L. (L.) donovani in the hamster using the standard primary visceral test procedure (Table I). Represented among these compounds were some natural products as well as some compounds known to be efficacious against other diseases. Although testing was done using several dosage levels as well as various routes of administration, as can be seen from Table I, only one of these compounds (BN97515) was noted to have any efficacy against visceral leishmaniasis in the hamster at any dosage level studied or when administered via any route. However, this compound as well as several of the compounds noted to be inactive against L. (L.) donovani in the hamster were toxic to the host, sometimes resulting in death (Table I).

The data obtained from this group of compounds indicates that none have any potential for further study in the chemotherapy of visceral leishmaniasis.

Two experiments were conducted to compare the toxicity to the host and antileishmanial efficacy against L. (L.) donovani of Amphotericin B, a compound with known efficacy, with that of selected analogs of this compound. As can be seen from Table II, the analog was not as active as Amphotericin B in either experiment. In the first experiment in which dosage levels of each were comparable, the parasite suppression was less in those hamsters receiving the analog. This lower activity of the analog was confirmed in the second experiment in which greater dosage levels of the analog were required to accomplish similar parasite suppression. Neither compound was toxic as tested.

A total of 4 cholesterol-lowering drugs (BP04971, Lescol; BP05003, Pravachol; BP05012, Zocor; and BP05021, Lamisil) were studied in hamsters for antileishmanial activity against L. (L.) donovani using the slightly modified primary visceral test system (Table III). None of these cholesterol-lowering compounds were active regardless of duration of treatment (i.e. up to 7 days prior to infection and continuing up to 14 days following infection) with dosage levels ranging from 182 to 4340 total mg/kg of compounds. None of these compounds were toxic to the hamster as studied.

Since none of these cholesterol-lowering compounds were suppressive when tested alone, an experiment was conducted to determine if BP14986 (Lipitor) administered at 182 total mg/kg dosage level combined with various dosage levels of BP05021 (Lamisil) i.e. 43.4, 434, or 4340 total mg/kg would be active (Table IV). Another combination involved Lipitor (BP19486) and Questran (BP09044) at dosage levels of 182 and 1456 total mg/kg respectively. Although these combinations were administered beginning 7 days prior to infection and continuing for 7 days after infection, none were active nor toxic.

A total of 4 cholesterol-lowering compounds (BP04999, Mevacor; BP04980, Lopoid; BP04962, Cholestid; and BP04944, Questran) were studied for activity against L. (L.) donovani in hamsters using mortality of the host as an indicator. The dosage levels used for each were 832, 416, and 208 and treatment began 3 days prior to infection and continued until death. No difference was noted in the mortality of treated groups when compared to the vehicle control.

Three of the same compounds studied for efficacy against L. (L.) donovani were tested for activity against cutaneous leishmaniasis caused by L. (V.) panamensis in hamsters (Table V). Although administration was done by different routes and at different dosage levels, none of these compounds had any efficacy against L. (V.) panamensis regardless of dosage level or route of administration used. As noted when studying these same compounds against L. (L.) donovani in hamsters, some of these were toxic to the host.

One compound, Baycol, was studied for activity against both L. (V.) panamensis and L. major in mice and was found to be inactive against either parasite as tested and not toxic to the host.

In the first experiment in which a topical preparation was evaluated for efficacy against cutaneous lesions caused by L. major lesions on mice receiving BN85873 had significantly closed and healed (94% suppression) by day 21 following completion of treatment and hair was growing back rapidly on the area previously covered by the ulcerated lesion (Table VI). By Day 35 following completion of treatment, all mice receiving the placebo were dead (measurement given on table was taken from lone mouse which was dead) while those treated with BN85873 showed 100% suppression of lesion size.

By day 56 following treatment one of six mice treated with BN85873 had a relapse consisting of a 30 mm² lesion approximately 1.0 cm from the original injection point. By Day 61, this lesion had grown to 50 mm² and remained this size until the termination of the experiment on Day 70. The lesion was removed and touch impression revealed the presence of amastigotes of Leishmania.

In a second experiment in which four topical preparations in which water concentrations were varied were evaluated for efficacy against cutaneous leishmaniasis caused by L. major (Table VII), all concentrations of WR279396 were equally suppressive by 3 weeks post treatment and only marginal differences in the percent suppression occurred at one or two weeks post treatment (range 77.0-86.0; 94.0-98.0 respectively).

Death of some of the treated mice occurred two days following completion of treatment. Three of ten mice which had received BP15269 and one of ten mice receiving BP15278 died. Significant weight loss was also noted in the group receiving BP15269 (-19%).

A total of 178 biopsy cultures obtained by WRAIR from 129 patients (both human and dog) were received by this laboratory for injection into hamsters and/or mice via various routes to assist in the confirmation of the presence or absence of protozoan blood parasites, to assist in determination of the type of protozoan parasite present, and to determine the susceptibility of laboratory animals (especially hamsters) to the parasites. A summary of the results is presented in Table VIII.

A total of 160 cultures representing 121 patients was injected intracutaneously into hamsters. Of these, 40 cultures representing 38 patients (all human) produced cutaneous lesions in hamsters generally ranging from 5-15 mm in diameter. Microscopic examination of microscope slide impressions of these lesions revealed the presence of amastigotes of Leishmania. When the lesions were homogenized and cultured in Schneider's Drosophila culture medium, promastigote stages of Leishmania were observed. These observations confirm the presence of cutaneous species of Leishmania in 38 patients. Furthermore, hamsters are susceptible to these Leishmania and the clinical disease observed in hamsters is similar to that observed in some established hamster-Leishmania model systems that are being used currently for chemotherapy studies (Hanson, et al., 12). Thus a laboratory animal model system is now in place that can be used to perform drug sensitivity studies with these recent isolates of cutaneous Leishmania as well as for experiments to develop new antileishmanial drugs for any of these that may be resistant to drugs currently being used for treatment.

A total of 47 cultures representing 35 patients was injected intracutaneously into mice (Table VIII). Of these, 4 cultures representing 3 patients produced cutaneous lesions in mice. One of these 3 cultures failed to produce infections in hamsters. The latter observation emphasizes the importance of using more than one laboratory animal species when attempting to diagnose infections of Leishmania in patients.

Injection of samples of selected cultures into hamsters and mice via the intracardiac route was done to determine whether the cultures contained any visceral species of Leishmania since this is the preferred route of infection of laboratory animals with these species (23). A total of 67 cultures representing 47 patients were injected into hamsters via the intracardial route. As can be seen from Table VIII, 13 cultures representing 8 patients injected into hamsters via this route produced visceral infections in hamsters. These observations strongly suggest the presence of visceral species of Leishmania in these cultures and therefore the patients from which they were taken.

A total of 24 cultures representing 17 patients were injected into mice via the intracardial route. None of the mice injected with these cultures via the intracardiac route became infected. This emphasizes the fact that the hamster is a much better animal

for use in attempting to diagnose visceral leishmaniasis in patient samples.

Injection of samples of selected cultures into mice via the intraperitoneal route was done primarily to determine whether the cultures and thus the patients from which they were taken contained any parasites of the genus Trypanosoma. None of the mice injected with either of these cultures developed demonstrable infections with Trypanosoma.

Biochemical characterization (typing) of these Leishmania in other laboratories (Jackson, personal communication) has verified that the cultures received by us and have produced cutaneous lesions in hamsters in our laboratory include Leishmania (V.) panamensis, L. (V.) braziliensis, and L. (L.) mexicana. Results of the typing studies also indicated that no difference can be detected between the parasites cultured from hamster lesions and those in the original cultures thus enhancing the usefulness of these strains of Leishmania in future chemotherapy and other types of studies.

Conclusions

1. The probability of identifying new compounds with antileishmanial activity and little or no host toxicity is low.
2. The lack of success encountered in this work in identifying new, effective antileishmanial drugs emphasizes the need for continued studies of possible potentially active new compounds.
3. Leishmanial cutaneous lesion development in golden hamsters is very helpful, along with other procedures, for the confirmation of the presence of Leishmania in biopsy obtained by WRAIR from patients with suspected infections with these parasites.
4. Visceral leishmanial infections in hamsters is helpful for the confirmation of the presence of visceral Leishmania in biopsy cultures obtained by WRAIR from patients with suspected infections.
5. The topical ointment WR279396 was very efficacious in the treatment of experimental cutaneous leishmaniasis caused by Leishmania major in Balb/c mice.
6. The infection of golden hamsters with several species of Leishmania of human origin (identified in other laboratories by biochemical typing as Leishmania (Viannia) panamensis, Leishmania (Leishmania) mexicana, and Leishmania (Viannia) braziliensis) resulted in clinical disease similar to that seen in human beings. Thus a laboratory animal model is now in place for the testing of promising new antileishmanial drugs against new isolates of Leishmania, some of which had various degrees of susceptibility to current therapeutic drugs.

Literature Cited

1. Kinnamon, K.E., E.A. Steck, P.S. Loizeaux, L.D. Hendricks, V.B. Waits, W.L. Chapman, Jr., and W.L. Hanson. 1979. Leishmaniasis: Military significance and new hope for treatment. Mil. Med. **44** (10): 660-664.
2. Tropical Disease Research, Seventh Programme Report, 1 January 1983 - 31 December 1984. UNDP/World Bank/WHO Imprimerie A. Barthelemy, Avignon, France 1985. Pages 7/3-7/18.
3. Chapman, W.L., Jr. and W.L. Hanson. 1984. Leishmaniasis. IN Clinical Microbiology and Infectious Diseases of the Dog and Cat. W.B. Saunders Company, Philadelphia. pp 764-770.
4. Most, H. 1968. Leishmaniasis. In: Internal Medicine in World War II. Volume III. Infectious Diseases and General Medicine. Office of the Surgeon General, Department of the Army, Washington, D.C.. pp. 1-48.
5. Naggan, L., A.E. Gunders, R. Dizian et al. 1970. Ecology and attempted control of cutaneous leishmaniasis around Jericho, in the Jordan Valley. J. Infect. Dis. **212**: 427-432.
6. Walton, B.C., D.A. Person, and R. Bernstein. 1968. Leishmaniasis in the U.S. Military in the Canal Zone. Am. J. Trop. Med. Hyg. **17** (1): 19-24.
7. Takafuji, E.T., L.D. Hendricks, J.L. Daubek, K.M. McNeil, H.W. Scagiola, and C.L. Diggs. 1980. Cutaneous leishmaniasis associated with jungle training. Am. J. Trop. Med. Hyg. **29** (4): 516-520.
8. Albrecht, H., I. Sobottka, C. Emminger, H. Jablonoski, G. Just, A. Stoehr, T. Kubin, B. Salzberger, T. Lutz, and J. Van Luzen. 1996. Visceral leishmaniasis emerging as an important opportunistic infection in HIV-infected persons living in areas nonendemic for Leishmania donovani. Arch. Pathol. Lab. Med. **120**: 189-198.
9. Estambale, B.B. and R. Knight. 1992. Protozoan infections and HIV-1 infection: a review. East Afr. Med. J. **69**: 373-377.
10. Peters, B.S., D. Fish, R. Golden, D.A. Evans, A.D. Bryceson, and A.J. Pinching. 1990. Visceral leishmaniasis in HIV infection and AIDS: clinical features and response to therapy. Q. J. Med. **77** (283): 1101-1111.
11. Rocha, P.S., L. Rebocho, L. Alvoeiro, G. Lourenco, R. Branco, R.M. Victorio, and M.C. de Moura. 1993. Visceral leishmaniasis and HIV infection: the diagnostic and therapeutic problems. Acta. Med. Port. **6**: 543-546.

12. Hanson, W.L., W.L. Chapman, Jr., and K.E. Kinnamon. 1977. Testing of drugs for antileishmanial activity in golden hamsters infected with Leishmania donovani. Internat'l. J. Parasitol. 7: 443-447.
13. Chapman, W.L., Jr., W.L. Hanson, and L.D. Hendricks. 1981. Leishmania donovani in the owl monkey (Aotus trivirgatus). Trans. Roy. Soc. Trop. Med. Hyg. 7: 124-125.
14. Chapman, W.L., Jr., and W.L. Hanson. 1981. Visceral leishmaniasis in the squirrel monkey (Saimiri sciureus). J. Parasitol. 67: 740-741.
15. Madindou, T.J., W.L. Hanson, and W.L. Chapman, Jr. 1985. Chemotherapy of visceral leishmaniasis (Leishmania donovani) in the squirrel monkey (Saimiri sciureus). Ann. Trop. Med. Parasitol. 79: 13-19.
16. Hanson, W.L., W.L. Chapman, Jr., V.B. Waits, and J.K. Lovelace. 1991. Development of Leishmania (Viannia) panamensis lesions and relationship of numbers of amastigotes to lesion area on antimony-treated and untreated hamsters. J. Parasitol. 77 (5): 780-783.
17. Kinnamon, K.E., E.A. Steck, P.S. Loizeaux, W.L. Hanson, W.L. Chapman, Jr., and V.B. Waits. 1978. The antileishmanial activity of lepidines. Am. J. Trop. Med. Hyg. 27 (4): 751-757.
18. Alving, C.R., E.A. Steck, W.L. Chapman, Jr., V.B. Waits, L.D. Hendricks, G.M. Swartz, Jr., and W.L. Hanson. 1980. Liposomes in leishmaniasis: Therapeutic effects of antimarial drugs, 8-aminoquinolines, and tetracycline. Life Sciences 26: 2231-2238.
19. Berman, J.D., W.L. Hanson, W.L. Chapman, Jr., C.R. Alving, and G. Lopez-Berestein. 1986. Antileishmanial activity of liposome-encapsulated amphotericin B in the hamster and monkey. Antimicrobial. Agents and Chemother. 30: 847-851.
20. Berman, J.D., W.L. Hanson, J.K. Lovelace, V.B. Waits, J.E. Jackson, W.L. Chapman, Jr., and R.S. Klein. 1987. Activity of purine analogs against Leishmania donovani *in vivo*. Antimicrobial. Agents and Chemother. 31 (1): 111-113.
21. Berman, J.D., G. Ksionski, W.L. Chapman, Jr., V.B. Waits, and W.L. Hanson. 1992. Activity of Amphotericin B Cholesterol Dispersion (Amphocil) in Experimental Visceral Leishmaniasis. Antimicrobial. Agents and Chemother. 36 (9): 1978-1980.
22. Croft, S.L. 1988. Recent developments in the chemotherapy of leishmaniasis. Trends Pharmacol. Sci. 9: 376-381.
23. Stauber, L.A. 1958. Host resistance to the Khartoum strain of Leishmania donovani. The Rice Institute Pamphlet Vol. XLV (1): 80-96.

24. Stauber, L.A., E.M. Franchino, and J. Grun. 1958. An eight-day method for screening compounds against Leishmania donovani in the golden hamster. J. Protozool. 5: 269-273.
25. Hendricks, L.D., D. Wood, and M. Hajduk. 1978. Hemoflagellates: Commercially available liquid media for rapid cultivation. Parasitol. 76: 309-316.
26. Hanson, W.L. and E.L. Roberson. 1974. Density of parasites in various organs and the relation to number of trypomastigotes in the blood during acute infections of Trypanosoma cruzi in mice. J. Protozool. 21: 512-517.
27. Wilson, H.R., B.W. Dieckmann, and G.E. Childs. 1979. Leishmania braziliensis and Leishmania mexicana: Experimental cutaneous infections in golden hamsters. Exptl. Parasitol. 47: 270-283.

APPENDIX

Table I. Summary of the studies of selected compounds against *L. donovani* in the golden hamster.

<u>Bottle Number</u>	<u>Dosage (TMK*)</u>	<u>Route</u>	<u>Percent Suppression</u>
BN50736	832	PO	- 6
	208		-11
	52		-76
	832	PO	17
	208		0
	52		- 1
BN52829	320	IM	<-29
	52		<-15
	320	PO	-12
	52		<- 9
BN55339	320	PO	60
	52		44
	320	IP	46
	52		44**
BK73690	104	PO	0
	26		- 5
BK94984	208	PO	27
	52		12
	13		0
BL00049	208	PO	3
	52		- 7
	13		3
	208	SQ	15
	52		8
	13		- 5
BL03899	208	PO	- 5
	52		- 9
	13		1
	208	SQ	ND***
	52		5
	13		- 8
BL05357	208	PO	21
	52		19
	13		- 7

<u>Bottle Number</u>	<u>Dosage (TMK*)</u>	<u>Route</u>	<u>Percent Suppression</u>
BL05366	208	PO	21
	52		- 7
	13		- 7
	208	SQ	34
	52		8
	13		9
BL07637	208	PO	26
	52		-15
	13		-15
	208	SQ	10
	52		8
	13		-16**
BL07646	208	PO	5
	52		16
	13		8
	208	IM	49
	52		31
	13		16**
BL08223	208	PO	12
	52		1
	13		9
	208	IM	27
	52		39
	13		17**
BL09033	208	PO	- 9
	52		- 2
	13		- 2
	208	IM	45
	52		17
	13		11**
BL09462	208	PO	13
	52		25
	13		16
BL11131	208	PO	16
	52		29
	13		3

<u>Bottle Number</u>	<u>Dosage (TMK*)</u>	<u>Route</u>	<u>Percent Suppression</u>
ZP55109	208	PO	17
	52		17
	13		19
	208	IM	27
	52		39
	13		17**
BN76829	208	IM	19
	52		13
	13		11
BN79526	208	IM	30
	52		29
	13		16
BN87635	208	IM	32
	52		21
BN97480	208**	IM	44
	52		- 1
BN97499	208	IM	- 8
	52		-11
BN97506	208	IM	23
	52		3
BN97515	208**	IM	70
	52		53
BP13934 [#]	168	PO	11
	42		14
BP13943 [#]	168	PO	32
	42		18
BP13952	208	PO	25
	52		18
BP13970	208	PO	- 3
	52		- 5
BP13961	208	PO	9
	52		10

PO: per os
IM: intramuscular
IP: intraperitoneal
SQ: subcutaneous

* Total mg/kilogram

**Toxic as indicated by death and/or greater than 15% loss of body weight.

* Hamsters receiving this compound were dosed for seven consecutive days.

Table II. Comparison of the suppressive activity of Amphotericin B and analogs against *L. donovani* in the golden hamster.

<u>Experiment No.</u>	<u>Treatment</u>	<u>TMK*</u>	<u>Percent Suppression</u>
1	Amphotericin B (BM16033)	3.0	100
		1.5	96
		0.5	94
		0.16	62
	Amphotericin B Analog (BN87902)	3.0	82
		1.5	70
		0.5	37
		0.16	20
2	Amphotericin B (BM16033)	3.0	100
		1.5	100
		0.5	96
	Amphotericin B Analog (BN91568)	24.0	100
		12.0	100
		6.0	97

*Total milligrams/kilogram body weight

Table III. Summary of studies of cholesterol-lowering drugs against L. donovani in hamsters.

<u>Treatment</u>	<u>TMK*</u>	<u>Percent Suppression</u>
Lescol (BP04971)	728	9
	364	3
	182	- 3
Pravachol (BP05003)	728	9
	364	7
	182	11
Zocor (BP05012)	728	1
	364	2
	182	-17
Lamisil (BP05021)	728	- 3
	364	-17
	182	-14

*Total milligrams/kilogram given for 7 days (3 days prior to infection and 4 days following infection).

Table IV. Summary of studies of combinations of cholesterol-lowering drugs against *L. donovani* in hamsters.

<u>Treatment Suppression</u>	<u>TMK*</u>	<u>Percent</u>
Lamisil (BP05021) alone	4340	25
Atorvastatin (Lipitor, BP05021) alone	182	17
Lamisil + Atorvastatin	43.4 182	9
Lamisil + Atorvastatin	434 182	-10
Lamisil + Atorvastatin	4340 182	- 1
Atorvastatin + Questran (BP04944)	182 1456	-18

*Total milligrams/kilogram given over a 14 day period (7 days prior to infection, the day of infection, and 6 days post infection).

Table V. Summary of the suppressive activity of selected compounds against *L. (Viannia) panamensis* in the golden hamster.

<u>Bottle Number</u>	<u>Dosages (TMK)</u>	<u>Route</u>	<u>Percent Suppression</u>
BN50736	832	PO	-47
	208		-58
	52		-44*
BN52829	320	IM	32
	52		30*
	320	PO	8
	52		5*
BN55339	320	PO	- 9
	52		5
	320	IP	51
	52		37

PO: per os

IM: intramuscular

IP: intraperitoneal

* Toxic as indicated by 15% loss of body weight or death.

Table VI. Comparison of the suppressive activity of BN85864 (Placebo) and BN85873 against Leishmania major in the Balb/c mouse.

<u>Day Following Treatment</u>	<u>Mean Lesion Size (mm²)</u>		<u>Percent Suppression</u>
	<u>BN85864</u>	<u>BN85873</u>	
0*	275.0	225.0	19.0
7	233.3	158.3	33.0
14	300.0	77.5	75.0
21	283.3	19.7	94.0
28	300.0	9.2	97.0
35	400.0	5.0	99.0
50	All Dead	0.0	100.0
61	--	0.0 [#]	100.0
70	--	0.0 [#]	100.0

*Completion of treatment

[#]Relapse in one mouse whose lesion measured 50 mm² 1.27 cm to the right of the original inoculation point.

Table VII. Summary of the suppressive activity of BP15287,
 BP15241, BP15250, BP15269, and BP15278 on L. major in
 the Balb/c mouse.

<u>Rx</u>	Week 1*		Week 2*		Week 3*	
	<u>Size</u>	<u>% Supp.</u>	<u>Size</u>	<u>% Supp.</u>	<u>Size</u>	<u>% Supp.</u>
BP15287	120.0	ND	157.5	ND	192.5	ND
BP15241	28.5	77.0	5.9	97.0	0.4	100.0
BP15250	25.0	80.0	4.5	98.0	1.0	100.0
BP15269	20.0	84.0	10.86	94.0	0.0	100.0
BP15278	17.2	86.0	6.67	96.0	0.0	100.0

*Weeks following completion of treatment.

ND: Not Done. This was the placebo or negative control group.

Size: Mean Lesion size in mm².

Table VIII. Laboratory animal confirmation of the presence of Leishmania from cultures obtained by WRAIR from patients with suspected infections.

<u>Culture Designation</u>	<u>No. of Cultures</u>	<u>No. Positive*</u>			<u>No. Positive*</u>		
		<u>Hamsters</u>	<u>IC*</u>	<u>IP*</u>	<u>Mice</u>	<u>IC*</u>	<u>ID*</u>
KT-1944	1	0/4				0/2	
MG-1108	1	0/4				0/3	
MG-0706	1	0/4			0/4		0/3
DW-4351	1	0/4	0/4				0/4
IW-7867	2	0/4 0/4					
DP-2750	2	0/4 0/4			0/4 0/4		
TG-8955	1	0/4			0/4		
GL-00117	1				4/4		
WR2159	1				4/4		
TP-9111	1	0/4					
WR2158	1				4/4		
MH-6147	7				0/4 0/4 0/4 0/4 0/4 0/4 0/4		
WR2156	1				0/4		
WR2165	2				1/4 0/4		
WR2164	1				4/4		
WR2157	1				1/4		
WR2160	1				4/4		
WR2162	1				4/4		

<u>Culture Designation</u>	<u>No. of Cultures</u>	<u>No. Positive*</u> <u>Hamsters</u>			<u>No. Positive*</u> <u>Mice</u>		
		<u>IC*</u>	<u>IP*</u>	<u>ID*</u>	<u>IC*</u>	<u>ID*</u>	<u>IP*</u>
WR2169	2				4/4 4/4		
JJ-4003	1				0/4		
WR2172H	1				1/4		
JG-1464	1				0/4		
PO-4048	1				0/4		
WA-8771	1	0/4			0/4		
RC-4977	1				0/4		
MJ-9322	1				0/4		
CE-2984	3				0/4 0/4 0/4		
AR-5950	1				0/4		
ES-6474	1				0/4		
TM-6684	1				0/4		
WR2174, WR2175, WR2176	3				4/4 4/4 4/4		
CC-4003	1				0/4		
WR2170	1				4/4		
TB-4927	1				0/4		
JK-2970	1	0/4			0/4		
DB-7413	2				0/4 0/4		
WR2177	1	4/4			0/4		
WR2178	1	4/4			0/4		
WR2180	1				0/4		
WR2179	1				0/4		
AH-7631	1				0/4		

<u>Culture Designation</u>	<u>No. of Cultures</u>	<u>No. Positive[#]</u> <u>Hamsters</u>			<u>No. Positive[#]</u> <u>Mice</u>		
		<u>IC*</u>	<u>IP*</u>	<u>ID*</u>	<u>IC*</u>	<u>ID*</u>	<u>IP*</u>
LV-8134	1				1/4		
HY-5897	2				0/4 0/4		
WR2182	1				4/4		
WR2183	1				0/4		
MC-5916	1	0/4					
AS	1	0/4			0/4		0/3
AA	1	0/4			0/4		0/3
BD	1	0/4			0/4		0/3
BI	1	0/4				0/3	0/3
BE	3	0/4 0/4 0/4			1/4 3/4 4/4	0/3	0/3
BJ	1				4/4		
BP	1				4/4	2/3	
BB	1				4/4		
B	1	0/4			0/4	0/3	
BJ	1				3/4		
BD	2				0/4 0/4		
BT	1				0/4		
BD	1	0/4			0/4	0/3	
CR	1	0/4			0/4	0/3	0/3
CM	4				2/4 1/4 0/4 0/4		
CL	1				0/4		

<u>Culture Designation</u>	<u>No. of Cultures</u>	<u>No. Positive*</u>			<u>No. Positive*</u>				
		<u>Hamsters</u>	<u>IC*</u>	<u>IP*</u>	<u>Mice</u>	<u>IC*</u>	<u>ID*</u>		
CB	1				3/4				
CO	1	0/4			0/3 0/3				
CJ	1				1/4				
CB	1				1/4				
DL	1	0/4			0/4	0/4	0/3		
DW	1				0/4				
DJ	2	0/4			0/4	0/3			
					0/4	0/3			
DL	2	0/4 4/4			0/4 0/4	0/3	0/3		
DK	1	0/4			0/4				
Dog #1	1	4/4			0/4	0/3			
Dog #2	1	4/4			0/4	0/3			
DA	1				3/3				
EA	1				0/4	0/3			
EL	1				0/4	0/3			
FD	2				0/4 0/4	0/3			
FC	1	0/4			0/4	0/3	0/3		
FR	3				0/4 0/4 0/4				
FH	1				0/4				
GT	1	0/4							
GJ	1				4/4				
GM	1	0/4			0/4	0/3			
HC	1				2/6				
HI	1	0/4			0/4	0/3	0/3		

<u>Culture Designation</u>	<u>No. of Cultures</u>	<u>No. Positive*</u> <u>Hamsters</u>			<u>No. Positive*</u> <u>Mice</u>		
		<u>IC*</u>	<u>IP*</u>	<u>ID*</u>	<u>IC*</u>	<u>ID*</u>	<u>IP*</u>
HS	1	0/4		0/4	0/3	0/3	
HG	2			0/4 6/6			
HS	1			4/4			
HJ	1			0/4			
HA	1			0/4			
HR	1	0/4		0/4	0/4	0/4	
JH	1	0/4		0/4	0/3	0/3	
KK	1			0/4		0/3	
KJ	1	0/4		0/4			
LS	1			0/3			
MV	1	0/4		0/4		0/3	
Mars (Dog #3)	8	4/4 4/4 4/4 3/4 1/4 0/2 0/4 0/4			0/1 0/2 0/4 0/3 1/3		
MJ	1			0/4			
MG	1	0/4		0/4	0/3	0/3	
MW	1			0/4		2/3	
NM	1	0/4		0/4	0/3	0/3	
ND	1			1/6		0/3	
NJ	7	0/3 0/4 0/3 0/3 0/3 0/3 0/3		0/4 0/4 0/4 0/4 0/4 0/4 0/4	0/3 0/3 0/3 0/3 0/3 0/3 0/3	0/3 0/3 0/3 0/3 0/3 0/3 0/3	

<u>Culture Designation</u>	<u>No. of Cultures</u>	<u>No. Positive*</u>			<u>No. Positive*</u>		
		<u>Hamsters</u>	<u>IC*</u>	<u>IP*</u>	<u>Mice</u>	<u>IC*</u>	<u>ID*</u>
OR	1				4/4		
OJ	1				0/4		
PD	2				0/6 0/6		
PL	1				0/4		
PJ	1				2/6		
PE	2				0/4 0/4		
RA	1	0/4			0/4		
RM	1				1/4		
SA	1				0/4		
SE	1				0/4		
SF	2				2/6 0/4		
SA	2				3/4 0/4		
TP	1				2/4		
UJ	2				0/4 0/4		
VF	1				0/4		
VL	2	0/4			0/4	2/6	0/4
WD	1				0/4		0/3
WA	2				0/4 0/4		
WM	1	4/4			0/4		
WT	1				0/2		

<u>Culture Designation</u>	<u>No. of Cultures</u>	<u>No. Positive*</u>			<u>No. Positive*</u>		
		<u>Hamsters</u>	<u>IC*</u>	<u>IP*</u>	<u>Mice</u>	<u>IC*</u>	<u>ID*</u>
WJ	3		0/4		0/4		0/3
			3/4		0/4		
			4/4		0/4		
WR2321	1		0/4		0/4	0/3	0/3
WR2324	1		0/4		0/4	0/3	0/3
YH	2				0/4		
					0/4		

* Positive for Leishmania

*IC: Cultures injected via the intracardiac route

*IP: Cultures injected via the intraperitoneal route

*ID: Cultures injected via the intradermal route